(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 January 2001 (04.01.2001)

PCT

(10) International Publication Number
WO 01/00854 A2

(51) International Patent Classification[7]: C12N 15/62, C07K 19/00, 14/705, 14/71, A61K 38/17

(21) International Application Number: PCT/GB00/02449

(22) International Filing Date: 26 June 2000 (26.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9914650.8 24 June 1999 (24.06.1999) GB

(71) Applicant *(for all designated States except US)*: **ANGIOGENE PHARMACEUTICALS LIMITED** [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SX (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **DAVIS, Peter, David** [GB/GB]; 10 Aston Park, Aston Rowant, Watlington, Oxfordshire OX9 5SW (GB).

(74) Agents: **WALTON, Seán, M.** et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/00854 A2

(54) Title: CHIMERIC PROTEINS MEDIATING TARGETED APOPTOSIS

(57) Abstract: Chimeric cell-surface proteins are described which may be used in the selective induction of apoptosis in particular target cell types such as cancer cells *in vivo* or *in vitro*. Nucleic acid sequences encoding such proteins and methods of use relating to cancer and other therapies are provided.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/GB 00/02449

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/62 C07K19/00 C07K14/705 C07K14/71 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, SCISEARCH, EMBASE, MEDLINE, CHEM ABS Data, BIOTECHNOLOGY ABS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MA J ET AL: "Second generation apoptotic-induced drug delivery system based on cells that express a flk-1:fas fusion protein." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 39, March 1998 (1998-03), page 277 XP000971899 89th Annual Meeting of the American Association for Cancer Research;New Orleans, Louisiana, USA; March 28-April 1, 1998, March, 1998 ISSN: 0197-016X the whole document --- -/--	1-19,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 2001	06.02.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 02684 A (UNIV LELAND STANFORD JUNIOR ;HARVARD COLLEGE (US)) 26 January 1995 (1995-01-26)	1-7, 11-21
A	abstract; claims 1-6 page 26, paragraph 2 -page 29, paragraph 1 page 30, paragraph 3 -page 32, paragraph 3 ---	8-10
X	RUDERT F ET AL: "APOPTOSIS IN L929 CELLS EXPRESSING A CD40/FAS CHIMERIC RECEPTOR: DISSOCIATION OF STIMULATORY FROM INHIBITORY DEATH SIGNALLING FUNCTIONS" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,US,ACADEMIC PRESS INC. ORLANDO, FL, vol. 204, no. 3, 15 November 1994 (1994-11-15), pages 1102-1110, XP002070355 ISSN: 0006-291X	1-7, 11-21
A	the whole document ---	8-10
X	RUDERT FRITZ ET AL: "Apoptosis through CD95 (Fas/APO-1), but not a CD40/CD95 chimeric receptor, is inhibited by phorbol-12-myristate-13-acetate." DNA AND CELL BIOLOGY, vol. 16, no. 2, 1997, pages 197-205, XP000978716 ISSN: 1044-5498 the whole document ---	1-7, 11-21
Y	NAOR D ET AL: "CD44:STRUCTURE, FUNCTION, AND ASSOCIATION WITH THE MALIGNANT PROCESS" ADVANCES IN CANCER RESEARCH,ACADEMIC PRESS, LONDON,GB, vol. 71, 1997, pages 241-319, XP000952636 ISSN: 0065-230X page 261, line 17 - line 23 page 283, paragraph 3 -page 284, paragraph 2 ---	8-10
Y	LI RUIHONG ET AL: "Chimeric CD4/CD44 molecules associate with CD44 via the transmembrane region and reduce hyaluronan binding in T cell lines." EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 28, no. 6, June 1998 (1998-06), pages 1745-1754, XP000953332 ISSN: 0014-2980 the whole document ---	8-10
Y	WO 93 13210 A (SQUIBB BRISTOL MYERS CO) 8 July 1993 (1993-07-08) page 1 -page 3; figure 1; example 1 ---	8-10
	-/--	

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/GB 00/02449

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RAFFIONI SIMONA ET AL: "Effect of transmembrane and kinase domain mutations on fibroblast growth factor receptor 3 chimera signaling in PC12 cells: A model for the control of receptor tyrosine kinase activation." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 52, 25 December 1998 (1998-12-25), pages 35250-35259, XP002157209 ISSN: 0021-9258 abstract; figure 1 ---	8-10
Y	UENO HIROO ET AL: "An epidermal growth factor receptor-leukocyte tyrosine kinase chimeric receptor generates ligand-dependent growth signals through the Ras signaling pathway." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 34, 1995, pages 20135-20142, XP002157210 ISSN: 0021-9258 abstract; figure 1 page 20138, left-hand column, paragraph 2 -----	8-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 8-21 partly , 1-7 complete

   nucleic acid constructs encoding chimeric polypeptides with an extra-cellular domain derived ,in particular , from CD44 ,corresponding vector, host cell , isolated peptide, pharmaceutical composition , use of nucleic acid ,method of producing polypeptides and method for inducing apoptosis in vitro

2. Claims: 8-21 partly

   nucleic acid constructs encoding chimeric polypeptides with an extra-cellular domain derived from ICAM-1 ,corresponding vector, host cell , isolated peptide, pharmaceutical composition , use of nucleic acid , method of producing polypeptides and method for inducing apoptosis in vitro

3. Claims: 8-21 partly

   nucleic acid constructs encoding chimeric polypeptides with an extra-cellular domain derived from a receptor for the cytokine vascular endothelial growth factor (VEGF) , corresponding vector, host cell , isolated peptide, pharmaceutical composition , use of nucleic acid , method of producing polypeptides and method for inducing apoptosis in vitro

4. Claims: 8-21 partly

   nucleic acid constructs encoding chimeric polypeptides with an extra-cellular domain derived from a receptor for the platelet-derived growth factor (PDGF) , corresponding vector, host cell , isolated peptide, pharmaceutical composition , use of nucleic acid , method of producing polypeptides and method for inducing apoptosis in vitro

5. Claims: 8-21 partly

   nucleic acid constructs encoding chimeric polypeptides with an extra-cellular domain derived from a receptor for the epidermal growth factor (EGF) ,corresponding vector, host cell , isolated peptide, pharmaceutical composition , use of nucleic acid , method of producing polypeptides and method for inducing apoptosis in vitro

INTERNATIONAL SEARCH REPORT

.formation on patent family members

Inter ·onal Application No
PC1/GB 00/02449

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9502684 A	26-01-1995	AU 696991 B	24-09-1998
		AU 7336394 A	13-02-1995
		CA 2167282 A	26-01-1995
		CN 1130401 A	04-09-1996
		CZ 9600084 A	16-06-1999
		EP 0776359 A	04-06-1997
		FI 960165 A	26-01-1996
		HU 73100 A	28-06-1996
		JP 9503645 T	15-04-1997
		PL 312586 A	29-04-1996
		US 6011018 A	04-01-2000
		US 6054436 A	25-04-2000
		US 6063625 A	16-05-2000
		US 6046047 A	04-04-2000
		US 6043082 A	28-03-2000
		US 6140120 A	31-10-2000
		US 5834266 A	10-11-1998
		US 5869337 A	09-02-1999
		US 5994313 A	30-11-1999
		AU 690898 B	07-05-1998
		AU 6240394 A	29-08-1994
		EP 0804561 A	05-11-1997
		FI 953812 A	11-08-1995
		JP 8510896 T	19-11-1996
WO 9313210 A	08-07-1993	US 5861151 A	19-01-1999
		AT 190353 T	15-03-2000
		AU 678144 B	22-05-1997
		AU 3327293 A	28-07-1993
		CA 2123303 A	08-07-1993
		DE 69230762 D	13-04-2000
		DE 69230762 T	13-07-2000
		DK 619843 T	05-06-2000
		EP 0619843 A	19-10-1994
		ES 2143499 T	16-05-2000
		FI 942943 A	17-06-1994
		GR 3033474 T	29-09-2000
		IL 104117 A	27-12-1998
		JP 7502413 T	16-03-1995
		MX 9207420 A	01-06-1993
		NO 942320 A	17-06-1994
		NZ 245473 A	28-08-1995
		PT 101148 A,B	31-03-1994
		PT 619843 T	31-08-2000
		ZA 9209595 A	19-08-1993

PATENT COOPERATION TREATY

REC'D 20 JUL 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SMW/CP5861372		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/02449	26/06/2000	24/06/1999
International Patent Classification (IPC) or national classification and IPC C12N15/62		
Applicant ANGIOGENE PHARMACEUTICALS LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22/01/2001	18.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Buchet, A Telephone No. +49 89 2399 7401

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02449

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-50 as originally filed

Claims, No.:

1-17 with telefax of 21/06/2001

Drawings, sheets:

1/23-23/23 as originally filed

Sequence listing part of the description, pages:

1-38, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 17.

because:

   ☒ the said international application, or the said claims Nos. 17 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☒ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations
see separate sheet

Reference is made to the following documents:

D1: Proceedings of the American Association for Cancer Research Annual vol. 39, 1998, p 277
D2: WO 95/02684

- D1 reports a cDNA construction comprising the 5'-end of the Fas transmembrane and intracellular death domain fused to the 3'-end of the flk-1 extracellular domain (flk-1:Fas). This construct was transformed into CCL209 bovine pulmonary artery endothelial cells. Surface expression of the chimeric protein was followed by western blot analysis. It was further shown that this receptor is specifically activated in the presence of the vascular endothelial growth factor (VEGF), e. g. in the form of the culture supernatant of cells overexpressing this factor. The cell death of CCL209/flk-1:Fas was observed in a dose-response relationship with the quantity of VEGF acting as a ligand for the flk-1 receptor. This system offers therapeutic outcomes: either inhibition of angiogenesis by transformed cells serving as pseudo-receptors, or by tumor cell cytotoxicity via apoptotic-induced drug delivery.

- D2 discloses DNA constructs encoding chimeric receptors able to initiate apoptosis in a cell containing said chimeric protein following exposure to a multimeric ligand (claim 1). The engineered cells which elimination is controlled can be used in gene therapy: They can be introduced into an organism after their transformation *ex vivo*; Alternatively, the target cells of the host organism can be *in vivo* transfected with the DNA construct.

Re Item I
Basis of the report

This report is also established on the basis of pages 1 to 38 of the sequence listing (SEQ ID NOS: 1 to 22).

Re Item III
Non-establishm nt of opinion with r gard to nov lty, inv ntiv st p and

CLAIMS:

1. An isolated nucleic acid encoding a chimeric polypeptide comprising;

(i)an extra-cellular domain which binds multivalent ligand preferentially at the surface of a target cell relative to a non target cell,

(ii) a membrane spanning domain, and

(iii) a cytoplasmic domain which induces cell death in a target cell upon binding of the extra-cellular domain with the multivalent ligand.

2. A nucleic acid according to claim 1 wherein the multivalent ligand is preferentially expressed in the vicinity of the target cell

3. A nucleic acid according to claim 1 wherein the binding of the extra-cellular domain is preferentially activated at the surface of a target cell relative to a non target cell.

4. A nucleic acid according to any one of the preceding claims wherein the target cell is selected from tumour cells, endothelial cells, smooth muscle cells, fibroblasts and hemopoietic cells.

5. A nucleic acid according to any one of the preceding claims wherein the cytoplasmic domain comprises a "death domain" from a member of the Fas/TNFR family.

6. A nucleic acid according to claim 5 wherein the cytoplasmic domain comprises the cytoplasmic domain from a receptor protein which is member of the Fas/TNFR family.

7. A nucleic acid according to claim 6 wherein the receptor protein is Fas.

8. A nucleic acid according to any one of the preceding claims wherein the extracellular domain is a CD44, ICAM-1, VEGFR1/Flt-1, VEGFR2/KDR/Flk-1, VEGFR3/Flt-4, PDGFRα, PDGFRβ or EGF receptor extracellular domain.

9. A nucleic acid according to claim 8 encoding an amino acid sequence as shown in any one of Figures 2A, 2B, 3A, 3B, 10A to 10D and 11A to 11D.

10. A nucleic acid according to claim 9 having a nucleic acid sequence as shown in any one of Figures 2A, 2B, 3A, 3B, 10A to 10D and 11A to 11D.

11. An expression vector comprising a nucleic acid according to any one of claims 1 to 10 operably linked to a regulatory element.

12. An expression vector according to claim 11 wherein the regulatory element is functional in a target cell type and not functional in a non-target cell type.

13. An expression vector according to claim 11 wherein the regulatory element is inducible.

14. A host cell comprising an expression vector according to any one of claims 11 to 13.

15 An isolated polypeptide encoded by a nucleic acid according to any one of claims 1 to 10.

16. A pharmaceutical composition comprising a nucleic acid according to any one of claims 1 to 10 or an expression vector according to any one of claims 11 to 13 and a pharmaceutically acceptable excipient.

17. A nucleic acid according to any one of claims 1 to 10, an expression vector according to any one of claims 11 to 13 or a composition according to claim 16 for use in a method of treatment of cancer, autoimmune disease, inflammation, psoriasis or other condition requiring selective destruction of a particular cell type.

18. Use of a nucleic acid according to any one of claims 1 to 10, an expression vector according to any one of claims 11 to 13 or a composition according to claim 16 in the manufacture of a medicament for use in the treatment of cancer, autoimmune disease, inflammation, psoriasis or

other condition requiring selective destruction of a particular cell type.

19 A method of producing a polypeptide according to claim 15 comprising;

introducing a nucleic acid according to any one of claims 1 to 10 into a host cell,

causing or allowing expression of said nucleic acid to produce said polypeptide.

20. A method for inducing apoptosis in a target cell comprising;

introducing a nucleic acid according to any one of claims 1 to 10 into a target cell,

causing or allowing expression of said nucleic acid to produce a polypeptide; and,

contacting said polypeptide with a ligand which interacts with said polypeptide,

said interaction causing apoptosis in said target cell.

21. A method according to claim 19 or claim 20 wherein the method is in vitro.

industrial applicability

- For the assessment of the present claim 17 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item IV
Lack of unity of invention

- The objection concerning the lack of unity of invention was overcome by restricted the claims to a single novel and inventive concept: the product claims relating to the DNA and vectors of the invention are now in the first and second medical use formats.

Re Item V
Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Novelty:

1-1. The present application claims DNA encoding chimeric transmembrane receptors to be used in gene therapy. Their cytoplasmic domain is intended to induce cell death (claims 5 to 7), in response to ligand binding by the heterologous extracellular domain (claim 1). Particular receptors are selected as a source for the extracellular domain (claims 8 to 10) so that the corresponding ligand is available only for the target cells which death is wished (claim 4). This implies that :
i) the ligand is preferentially expressed in the vicinity of the target cell (claim 2) and/or
ii) the binding is only activated for the receptors of said target cells (claim 3).
The following examples are given: The hyaluronan binding ability of CD44 is specifically observed on malignant cells; ICAM-1 functions as a ligand for the β2-integrin LFA-1

which is exclusively expressed on hemopoietic cells and which ligand-binding function is only induced in particular conditions; The VEGF factor is known to be specifically produced by tumor cells in response to hypoxic conditions; The PDGF factor is associated with vascular smooth muscle cell proliferation; Very high levels of the EGF factor normally produced by epithelial cells are observed in malignancies.
DNA constructs (claims 1 to 10) and/or recombinant vectors (claims 11 to 13) for use in medical treatment (claims 15), pharmaceutical compositions thereof (claim 14) and their medical use (claims 16 and 17) are claimed.

1-2. D1 discloses a DNA construct which corresponds to one disclosed in the present claim 8. However, D1 does not intend to use such a nucleic acid or the corresponding recombinant vector in medical applications; the therapeutical outcomes mentioned in D1 are based on the whole cells expressing the recombinant receptors. Therefore, D1 does not anticipate the subject-matter of the present set of claims.

1-3. D2 mentions DNA constructs encoding chimeric receptors which induce apoptosis, possibly *in vivo* injected to a specific subset of cells from a patient. However, the selected extracellular domains do not have the essential technical feature disclosed in claim 1 and do not correspond to those exemplified in claim 8: In contrast, they have an uniform binding activity independent of the cell population. Therefore, D2 is not novelty-destroying for the claimed subject-matter.

1-4. For all these reasons, claims 1 to 17 fulfil the requirements of Article 33.2 PCT.

2) Inventive step:

- Even if the binding between some receptors and their ligands was known to be conditional, the present invention is the first disclosure reporting the use of this property to obtain DNA constructs for use in gene therapy, which encode chimeric receptors initiating apoptosis specifically in the desired cells.

- Even if a corresponding DNA construct was already disclosed in the prior art (in particular in D1), there was no indication that such constructs could be used for the claimed therapeutical applications because of their property mnetioned above. The use

of the claimed constructs has evident benefit over the prior art (specificity, efficiency...).

- Therefore, claims 1 to 17 are considered to meet the requirements of Article 33.3 PCT.

3) Industrial applicability:

- Claim 17 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
SMW/CP5861372		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 02449	26/06/2000	24/06/1999
Applicant		
ANGIOGENE PHARMACEUTICALS LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[X] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02449

Box I Observations where certain claims were found unsearchable (Continuation of item 1 f first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 20 , af as it concerns an in vivo method ,is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[X] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.